Synthesis of molecular probes targeting ER flippases

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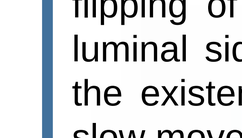

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Abstract: Here, the synthesis of lipid analogs for the purpose of assaying, capturing and isolation of candidate flippase proteins is described. For this purpose, we are synthesizing fluorescent mimics of the natural substrates, with an ω-terminal fluorophore (NBD).

Introduction: Most proteins that enter the secretory pathway become glycoproteins, i.e. they are modified by sugars, such as N-glycans. The absence of N-glycans is lethal, and alterations in glycosylation patterns may lead to devastating diseases, including cancer. The assembly of the canonical oligosaccharide donor for N-glycosylation requires the flipping of dolichol-containing glycolipids from the cytoplasmic to the luminal side of the endoplasmic reticulum (ER). This process implies the existence of proteins (flippases) that facilitate the otherwise very slow movement of polar glycolipids through the membrane (**FIGURE 1**).[1]

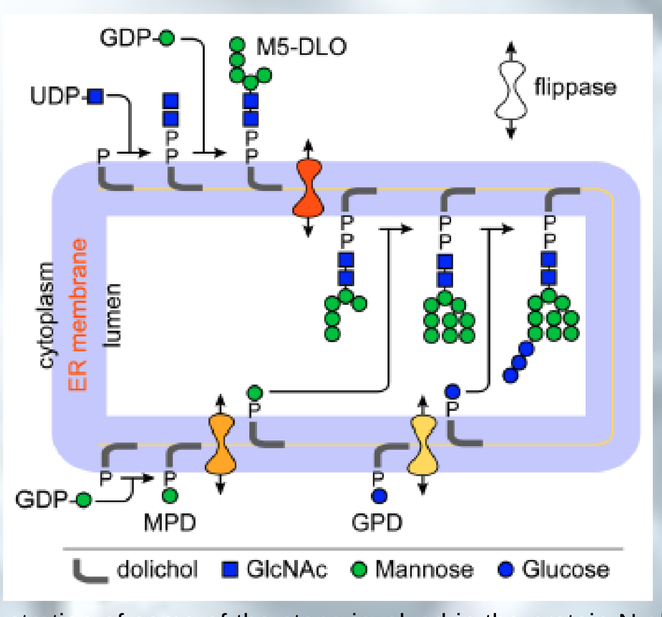


FIGURE 1 Illustration of some of the steps involved in the protein N-glycosylation pathway, located in the ER membrane.

The aim of this project is to synthesize a molecular probe, which mimics the natural structure (M5-DLO) (**FIGURE 2A**), with the addition of a fluorescent tag (NBD) on the ω-terminal of the dolichol chain (**FIGURE 2B**), which should serve as tool for the identification of the proposed ER flippases. These modifications regard mostly the hydrophobic moiety of the entire structure.

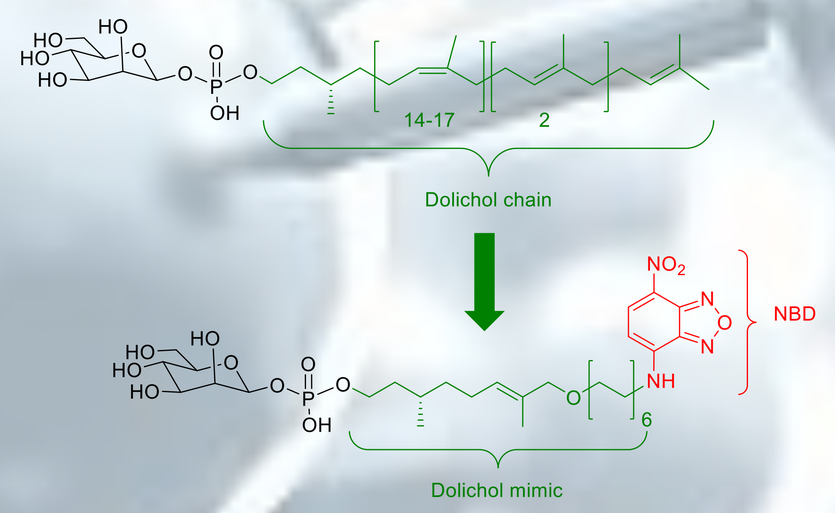
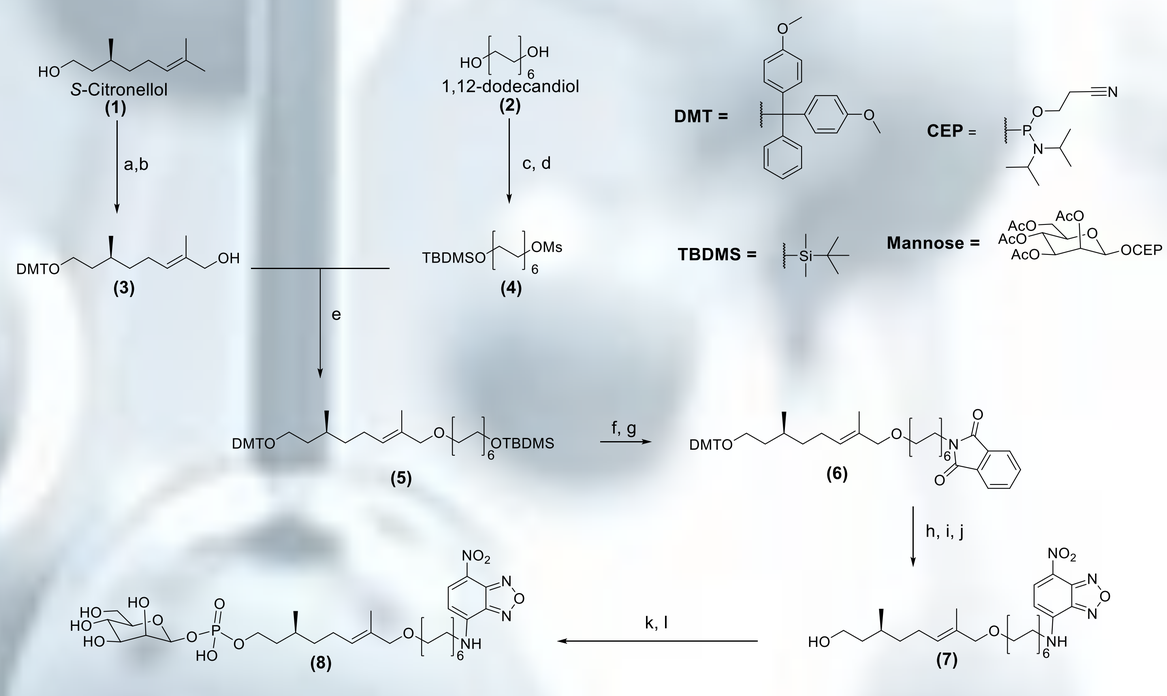


FIGURE 2 Structure of the natural substrate (A) and the corresponding fluorescent analogue (B).

Synthesis: This multistep synthetic route (**SCHEME 1**) starts with the allylic oxidation of protected S-citronellol (2), followed by coupling, with a protected and modified 1,12-dodecandiol (4), obtaining the relative product (5) through the ether bond formation. The conversion of hydroxyl group in amine, of the dodecandiol moiety, occurs by phthalimide formation as intermediate (6). The reduction of the latter leads to the amine functional group as product which is, then, coupled to NBD (7). The DMT protecting group is cleaved from citronellyl part and the obtained hydroxy group reacts with β-CEP-2,3,4,6-tetraacetylmannopyranoside, leading to relative product (8).



SCHEME 1 Reagent and conditions: a) DMT-Cl, Pyridine, 80°C; b) SeO_2, Pyridine/EtOH, then $NaBH_4$, EtOH; c) TBDMS-Cl, Imidazole, DMF; d) Ms-Cl, Et_3N, DCM, 0°C; e) NaH, DMF, 80°C; f) TBAF, THF; g) Phthalimide, PPh_3, DEAD, THF; h) $NH_2NH_2 \cdot H_2O$, THF; i) NBD-Cl, Et_3N, EtOAc/DCM; j) TCA 3% in DCM/MeOH (1:1); k) ETT, DCM, then tBuOOH; l) NH_3 in MeOH (2M)

Conclusion: The probe has been synthesized, and it will be used for both in vitro and in vivo studies. Based on the first tests, the alkyl chain can be subjected to further modification. All synthetic steps have been optimized, finding the best conditions in order to get the highest yield, especially when NBD is on the structure since it is unstable in basic environment for long times.

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Reference

[1] Sanyal, S., and Menon, A. K. (2009) Flipping lipids: why an' what's the reason for? *ACS Chem Biol 4*, 895-909